Evaluation of antiinflammatory properties and biosynthesis of copper nanoparticles from saltmarsh – an in-vitro study

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Abstract:

BACKGROUND: To synthesize copper nanoparticles utilizing the aqueous extract of Suaeda monoica plant (Salt marsh), a mangrove plant. The nanoparticles synthesized are of small size and high stability, leading to further analysis of the antibacterial activity of gram-positive (S.mutans) and gram-negative Klebsiella species, Salmonella typhi) bacterial strain using the disk diffusion method.

AIM: To analyze the antibacterial activity of the biologically synthesized copper nanoparticles against various pathogenic bacteria.

MATERIALS & METHOD: Aqueous extract synthesized from Suaeda monoica leaves, CuSO4 utilized as a precursor for copper nanoparticles synthesis. Characterization of NPs, done using UV Vis spectrophotometer. Further, a well diffusion test was done with different concentrations of copper nanoparticles (75µl and 100 µl), with various bacterial strains. The Zones of Inhibition was for the pathogens, with the positive control of Tetracycline and with negative control of methanol.

RESULTS: The average size of NP is 37 mm as the resonant peak absorbance wavelength is 570nm. The maximum of inhibition exhibited by Streptococcus mutans of 13.5mm at 100 µl dosage of copper nanoparticles and the standard antibiotic (Tetracycline) exhibited 22mm of ZOI.

CONCLUSION: The present study revealed that significant antibacterial activity is showcased by the biologically synthesized nanoparticles from the Suaeda monoica plant.

KEYWORDS: *Copper nanoparticles, Suaeda monoica, anti-inflammatory activity.*

INTRODUCTION:

There is an increasing commercial demand for metal nanoparticles due to their potential applicability in various areas such as electronics, catalysis, energy, textile, and medicine. Nanotechnology is becoming a key component of the biomedical and pharmaceutical industries.[1] Nanoparticles (NPs) are used for a variety of things, including biosensors, medicine administration, and diagnosis.[2] Different nanoparticles come in a variety of sizes, shapes, and compositions having distinctive physiochemical traits. As hazardous substances are not used as reducing and capping agents, which would cause the nanoparticles to aggregate, the biological production of nanoparticles is demonstrated to be both environmentally safe and cost-effective. Because it is very difficult to cultivate microbial cultures for an extended period and contamination is a possibility, biogenic NPs can be produced using plant phytochemicals (also known as bioactive substances).[3]

The Suaeda monoica plant, also called as Salt Marsh, is a species of the Amaranthaceous family and is native to the Arabian Peninsula and South India. Saltmarsh is isolated to areas with saline or alkaline soil which served as a supply of sodium carbonate in antiquity, which was used to make glass. Hepatitis has historically been treated with the leaf from S. monoica.[4,5]

A copper nanoparticle is a copper-based particle 1 to 100 nm in size. Like many other forms of nanoparticles, a copper nanoparticle can be prepared by natural processes or through chemical synthesis. The plant extract like salt marsh is an alternative green technology used to produce metal and metal oxide nanoparticles. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles through a single-step green synthesis process. Copper nanoparticles readily intermix and bind with other metals, ceramics, and polymers, and they exhibit physiochemical stability in the compounds. So they can be used for wide applications in dentistry.[6,7,8]

Anti-inflammatory drugs, also called anti-inflammatories, make up about half of analgesics. Copper nanoparticles have superior antibacterial properties when comparing them to present day antibiotics.[1,2] Extensive research is still carried out to identify the other biological properties of saltmarsh. A natural anti-inflammatory plant which can be used on a day-to-day basis. Literature available regarding synthesis of the copper nanoparticle from Suaeda monoica plant extract anti-inflammatory property are limited.
So, the aim of the present study is to synthesis of the copper nanoparticles forms salt marsh and evaluate its antibacterial efficacy.

MATERIALS AND METHOD:

EXTRACT PREPARATION

Suaeda monoica plant was grinded in the mortar and pestle for formation of the preprocessing powder. Further, the moisture removed by keeping the material used heat incubation to form a dry preprocessed powder (30 minutes, 37°C temperature). Then aqueous extract was prepared by adding 90ml of distilled water in 20g of salt marsh. The mixture was kept for incubation in the orbital shaker for 2 days (Crude extract synthesized).[9,10] Using whatman # 1 paper filtration of this material was done and the filtrate was preserved for nanoparticle synthesis (Figure 1a, b, c & d).

BIOSYNTHESIS OF COPPER NANOPARTICLES

Metal solution was formed using 20mg of Cu powder is dissolved in 90 ml of distilled water. To the material 10 ml of the plant extract was added and kept on an orbital shaker for 2 days.[11,12,13] (Figure 2a, b & c)

UV SPECTROPHOTOMETER

The reduction of copper sulphate to pure Cu nanoparticle was monitored by using ultraviolet visible spectrophotometer. UV-Vis spectral analysis was done using a double beam spectrophotometer in the range 400–700. The absorption spectrum of pale-yellow nanoparticle solution prepared with the proposed method showed a plasmon absorption band with a maximum of 57. Initial reading done before the synthesis of nanoparticles; but after 1 hour or after synthesis of nanoparticles (Synthesis time period), final reading taken (wavelength scanning done). This absorbance v/s wavelength graphical representation determined the optical absorption of copper nanoparticles in the wavelength region of 480 to 800 nm.[14,15]

ANTI INFLAMMATORY ACTIVITY

BSA (Bovine Serum Albumin) was used as the assay's reagent. In animal serum, BSA (bovine serum albumin) makes up more than 60% of all proteins. It is frequently used in cell culture, particularly when adding extra protein was necessary but the other serum components were undesired. BSA denatures and begins to express antigens when heated. The pH of the reaction mixture was raised to 6.8 using 1N HCL after being mixed with 2ml of 1 percent Bovine albumin fraction in 5 test tubes containing varying quantities (10–50 l) of saltmarsh extract. The reaction mixture was incubated at room temperature for 20 minutes in a water bath. The mixture was brought to room temperature before the absorbance at 660 nm was determined. The standard used was Aspirin sodium in various concentrations. Formula used for calculating the[11,16,17]

% Inhibition: % Inhibition = Control O.D – Sample O.D / Control O.D

A basic sign that copper nanoparticles have been synthesized and properly dispersed is the colouring difference of the plant extract from pale yellow to brown[1,2,18] The copper nanoparticles that were biosynthesized show potential anti-bacterial activity in a dose-dependent manner. In relation to the latter topic, earlier studies have supported the findings of the current study by indicating that DNA molecules with a high surface area to volume ratio and smaller size deactivate and interfere with bacterial colony reproduction. Additionally, compared to synthetic

nanoparticles, these organically produced copper nanoparticles are less hazardous. An increase in the produced extract's concentration indicates an increase in antioxidant activity. Increase in concentration of the prepared extract shows increased antioxidant activity. Saltmarsh is a potent anti-inflammatory agent as it shows nearly equal high anti-inflammatory activity compared to aspirin shown in. Phytochemicals like Anthocyanin is the reason for potent anti-inflammatory activity of saltmarsh. Quercetin and Kaempferol glycosides are other phytochemicals present in saltmarsh. Phytochemicals inhibit free radical formation thus preventing cells from undergoing necrosis or apoptosis. Saltmarsh has a good free radical scavenging property making it a potent anti-inflammatory agent.[19]

UV-Vis spectroscopy was used to confirm the absorbance of free radicals by the extract subjected to the inhibition of albumin denaturation assay to analyze its anti-inflammatory activity. The UV-Vis Spectra was recorded for the copper nanoparticles using salt marsh extract. It was observed from the spectra that the extract at 660 nm had the highest absorbance at a concentration of 50µl (92.2%), which was indicative for significant anti-inflammatory activity, as potent as diclofenac sodium itself. This confirms the potent efficacious anti-inflammatory activity of the copper nanoparticles synthesized using salt marsh extract (Figure 3, 4 and 5).[19,20]

DISCUSSION

In our present study, when subjected to a DPPH assay, Copper nanoparticles produced with salt marsh demonstrated the maximum absorbance at a concentration of 50µl (91.4%) when tested for anti-inflammatory action at a wavelength of about 660 nm when submitted to an inhibition of albumin denaturation assay.[21,22]

Devi BV et al., evaluated the anti-inflammatory efficacy of zinc oxide nanoparticles produced using grape seed extract and found that they are a powerful anti-inflammatory medication with few sides' effects. Another study done by Ethel Jeyaseela Jeyaraj et al., regarding the extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals.[23] The use of maceration or ultrasonic aided extraction considerably improved the production of phytochemicals from C. ternatea flowers (16–247% of increase). C. ternatea flowers have been used to separate phytochemicals such as kaempferol, quercetin, and myricetin glycosides, as well as anthocyanins. Clitoria ternatea flower extracts have been better to have antibacterial, antioxidant, anti-inflammatory, cytotoxic, and antidiabetic properties, all of which are advantageous to human health.[24] Extensive research needed to further explore more biological activities and can be implied in nano science to improve other properties.

In the present study, we tested the anti-inflammatory activities of copper nanoparticles produced with salt marsh in a similar way to these investigations. According to laboratory studies, the antioxidant properties of blue tea may aid to prevent DNA damage and inflammation.[8.9.10] As a result, salt marsh has anti-inflammatory properties due to its high flavonoid content. Salt marsh possesses antioxidant action, according to the studies described above, but there have been no experiments incorporating salt marsh into nanoparticles. Even though the present study suggests that salt marsh nanoparticles have anti-inflammatory properties, more clinical trials are needed to confirm these findings for clinical application.

CONCLUSION:

The present study suggests that salt marsh-mediated copper nanoparticles have good anti-inflammatory properties. It may be concluded that anti-inflammatory activity of salt marsh is not as effective as Aspirin, but it can be more effective when its concentration is increased to acceptable levels. As this is a preliminary study, further analysis of these nanoparticles over other various drugs is required.

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Author Contributions:

Akifa Beegum : Literature search, Data collection analysis, Manuscript drafting
Dr. Ramesh R: Data Verification, Manuscript draft

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Figure 1a: shows Salt marsh sample

Figure 1b: shows powdered sample

Figure 1c: Processing of extract

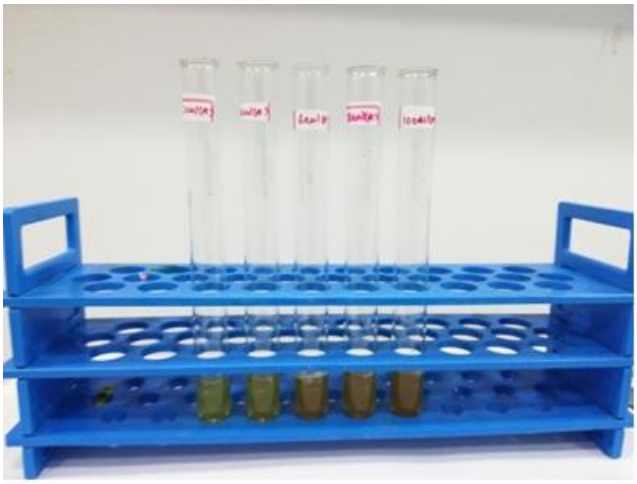
Figure 1d: Aqueous extract

Figure 1a, b, c & d shows preparation of aqueous extract

Figure 2a shows Copper sulphate solution

Figure2b: copper sulphate and sample (initial stage)

Figure 2c: shows final stage of synthesis



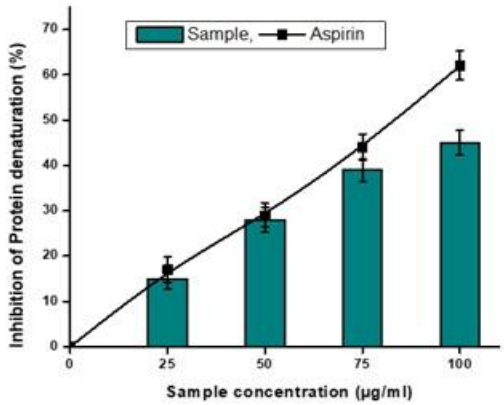


Figure 3 shows: antiinflammatory action of copper nanoparticles

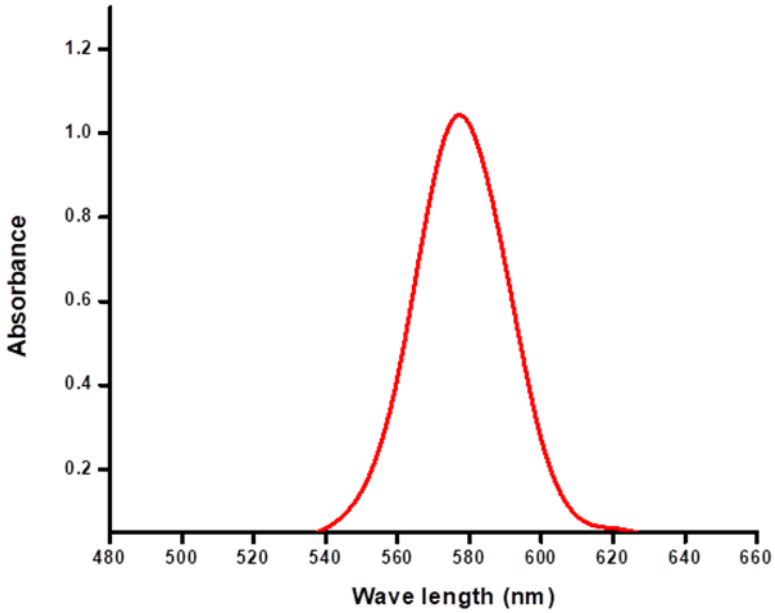


Figure 4 shows uv absorbance for antiinflammatory activity

µg/ml	Inbt.hmly	St.Er	Aspirin	St.Er
0	0	0	0	0
25	11	2	18	2.6
50	16	2.1	31	2.8
75	27	2.3	45	2.5
100	34	2.2	61	2.8

Table 1 shows antiinflammatory activity for different concentration